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| Cell line |  | G1 (\%) | S (\%) | G2/M (\%) | > 4N (\%) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{gathered} \text { MDA- } \\ \text { MB-231 } \end{gathered}$ | siCon | 43.56 | 32.98 | 19.57 | 2.10 |
|  | siDrp1 | 23.43 | 21.63 | 34.88 | 16.69 |
| $\begin{gathered} \text { MDA- } \\ \text { MB-157 } \end{gathered}$ | siCon | 40.05 | 32.36 | 17.12 | 5.30 |
|  | siDrp1 | 31.57 | 23.58 | 26.49 | 8.61 |
| A549 | siCon | 45.00 | 43.17 | 9.70 | 1.76 |
|  | siDrp1 | 50.53 | 32.26 | 14.22 | 2.05 |
| H1299 | siCon | 31.46 | 56.91 | 10.92 | 0.94 |
|  | siDrp1 | 41.70 | 35.24 | 19.71 | 1.28 |
| MCF-7 | siCon | 52.83 | 35.19 | 9.98 | 0.49 |
|  | siDrp1 | 54.84 | 26.57 | 14.20 | 1.02 |
|  | sip53 | 61.52 | 23.23 | 10.04 | 0.56 |
|  | $\begin{gathered} \text { sip53/ } \\ \text { Drp1 } \end{gathered}$ | 54.64 | 22.27 | 15.35 | 1.10 |

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Fig. S1. Both genetic interruption and pharmacological inhibition of Drp1 induce G2/M cell cycle arrest and aneuploidy. (A) siRNA-mediated knockdown of Drp1 induces G2/M cell cycle arrest and aneuploidy in various of cell lines. The human breast carcinoma cell lines MDA-MB-231 (p53R280K) and MDA-MB-157 (p53 null), and the human lung carcinoma cell lines A549 (p53 wt) and H1299 (p53 null) were transfected with control or Drp1 siRNA. Cells were collected at four days after siRNA transfection, and the cell cycle profile was assessed by flow cytometric analysis of BrdU and propidium iodide staining. (B) Breast carcinoma MCF7 (p53 wt) cells were transfected with control siRNA or siRNA targeting Drp1 and p53. Cell cycle profile was assessed as described in (A). (C) The percentage of cells in $\mathrm{G} 1, \mathrm{~S}, \mathrm{G} 2 / \mathrm{M}$ and $>4 \mathrm{~N}$ which were indicated in the square regions in (A) were quantified and summarized in the table. (D) Pharmacological inhibition of Drp1 by a small molecule inhibitor mdivi-1 induces G2/M cell cycle arrest and aneuploidy. MDA-MB-231 cells were treated with DMSO as vehicle or mdivi-1 for 48 hours with indicated concentrations. Cell cycle distribution was determined by flow cytometric analysis of propidium iodide stained cells. The percentage of the cells containing a DNA content of 4 N and $>4 \mathrm{~N}$ are indicated. Data presented in this figure are representative of three independent experiments.


Movie 1. Mitochondrial dynamics in a control MDA-MB-231 cell. MDA-MB-231 cells expressing pDsRed2-Mito and histone H2BGFP were transfected with control siRNA. Four-dimensional images were acquired (z-stack over time) using a Nikon A1 confocal microscope. DsRed (mitochondria) channel is shown.


Movie 2. Mitochondrial dynamics in a Drp1-deficient MDA-MB-231 cell. MDA-MB-231 cells expressing pDsRed2-Mito and histone H2B-GFP were transfected with Drp1 siRNA. Four-dimensional images were acquired (z-stack over time) using a Nikon A1 confocal microscope. DsRed (mitochondria) channel is shown.


Movie 3. Mitochondrial fission in a control MDA-MB-231 cell. A region in Movie 1, which is also indicated in Figure 5C was enlarged.


Movie 4. Suppressed mitochondrial remodeling in a Drp1-deficient MDA-MB-231 cell. A region in Movie 2, which is also indicated in Figure 5C was enlarged.


Movie 5. Mitochondrial branching event in a Drp1-deficient MDA-MB-231 cell. A region in movie 2, which is also indicated in Figure 5C was enlarged.


Movie 6. Generation of a net-like structure of mitochondria from a fork-like structure in a Drp1-deficient MDA-MB-231 cell. A region in movie 2, which is also indicated in Figure 5C was enlarged.

